A New Look at Insect Respiration[1]

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Abstract. A novel thermographic method has been used for simultaneously monitoring the passage of air through up to eight spiracles of endopterygote insects. Measurements on pupae of various lepidopteran species revealed active regulation of inspirations and expirations through one or two spiracles while the majority remained hermetically closed for prolonged periods. Because of subatmospheric hemocoelic pressure acting bellows-like on the large tracheae and air sacs, air is quickly sucked into the tracheal system whenever a spiracle opens. Large, mechanically produced positive peaks in hemocoelic pressure are associated with periodic outbursts of tracheal gases through specific spiracles. During the rhythmic pulsations in hemocoelic pressure, some spiracles open and close at different locations so that CO_2 is quickly ventilated. Spiracles on the same segment can function in synchrony with a spiracle on some other, even distant segment. In the period of subatmospheric hemocoelic pressure, the spiracles usually open in "twinkles" or flutters lasting only 50–300 ms. Some pupae, for example, diapausing *Manduca*, use only one "master spiracle" which opens for 200 ms about once a minute. Each opening is accompanied by a gulp of 500 nl of air sucked in by negative tracheal pressure. All other spiracles may be hermetically closed for 16 h or more.

It is concluded that insect respiration is controlled by a hitherto unknown, brain independent, neuromuscular mechanism (coelopulse) consisting of two main components: (a) a mechanism that integrates proprioceptive input to control the location and timing of the spiracular openings, and (b) a coordinated system of hemocoelic pressure control that regulates the force and direction of air flow through the spiracles. The results of this study question the general validity of the classical theory of insect respiration by simple gaseous diffusion.

Introduction

Flying or running insects show regular pumping movements of the abdomen. These movements are associated with rhythmic changes of internal body (hemocoelic, intratracheal, or hemolymph) pressure which, acting on the walls of the tracheal sacs and tubes, produce bulk-flow of gases through the spiracles. This type of ventilation resembles ventilation of mammalian lungs by the respiratory muscles of the chest and diaphragm. The basic features of such convective tracheal ventilation have been reviewed by Babák (1912), Buck (1962), Mill (1974), Miller (1974, 1981), Kaars (1981) and Kestler (1985). The innervation and regulation of the spiracles has been described best by Miller (1981).

More complicated conditions have been encountered in immobile resting stages having very low respiratory exchange. It was believed until now that these stages—such as diapausing pupae—show no respiratory movements. Oxygen was expected to penetrate within the body by simple diffusion through the spiracles while CO_2 diffused in the opposite direction. Though this so called "diffusional theory of insect respiration" is generally associated with the late August Krogh, it was actually proposed over 150 years ago (see Wigglesworth, 1984). What Krogh accomplished was the measurement and mathematical calculations of diffusion rates of respiratory gases through the tracheal system. Subsequently, the general validity of Krogh's work has stood the test of time (see Buck, 1962, and Kestler, 1985).

In 1967 Schneiderman and his colleagues (Levy and Schneiderman, 1966; Brockway and Schneiderman, 1967) showed that discontinuous respiration was associated with specific changes in mechanical pressure within the tracheal system. Later Sláma (1976) observed that

certain immobile stages of insects exhibited rhythmic pulsations in hemocoelic pressure. Provansal *et al.* (1977) emphasized that these pulsations, which are currently known as the extracardiac pulsations, might be associated with regulation of water balance and tracheal ventilation. More recently I have used special isotonic strain-gauge transducers (Sláma, 1984a) and microrespirographs (Sláma, 1984b) to document that insects can actively and selectively control the bulk flow of gases through the spiracles. The homeostatic control of hemocoelic pressure on tracheal ventilation appeared to be effected by a novel, brain-independent, cholinergic circuitry with the centers located in thoracic ganglia of the ventral nerve cord (Sláma *et al.*, 1979; Sláma, 1986). Such an autonomic, parasympathetic-like nervous system has recently been found in various species and developmental stages of insects. It has been called the coelopulse system (Sláma, 1988b). Encouraged by these findings I have worked since 1979 on a method that could display the dynamics of respiration through the spiracles. This paper describes some initial results obtained with a microanemometric network that can monitor over prolonged periods the gas flow through one or more spiracles.

Material and Methods

The pupae of all the investigated species were obtained from our laboratory cultures. Larvae of *Actias selene* were fed fresh *Rhododendron sp.* leaves. Eggs were purchased from dealers, and the pupae were kept and measured at 25°C. *Sphinx ligustri* larvae were fed fresh leaves of *Ligustrum vulgare* outdoors in September. Diapausing pupae were stored at 5°C; measurements were made at room temperature (24–25°C). *Hyalophora cecropia* larvae were reared on willow (*Salix caprea*) and *Manduca sexta* larvae on an artificial diet at 25°C. Eggs of the latter two species were obtained courtesy of Prof. L. M. Riddiford and Dr. K. Hiruma of the University of Washington, Seattle. Diapausing pupae of these species were stored at 5°C; measurements were made at room temperature.

The spiracles to be measured were permanently equipped with male parts of small, plastic connectors manufactured from disposable tips of common automatic pipettes (Pipetman). Female parts of the conical fittings were fixed to 250 mm long anemometric tubings (teflon tubing 0.6 mm I.D.; 1.0 mm O.D.) leading to a multiple anemometric transducer (Fig. 1B). The fittings over the spiracles were smeared with silicone grease to insure a tight seal and easy installation or removal. Originally, the hemocoel of the pupae was connected by a steel needle with a special hydraulic transducer (Sláma, 1976), which could record simultaneously all respiration-de-

pendent changes in hemocoelic pressure. It appeared, however, that the epidermal injury caused by penetration of the integument might cause long-lasting disturbances of respiratory functions, especially in diapausing pupae. After invention of a method for indirect recording of hemocoelic pressure from the body surface (Sláma, 1984a), the hydraulic transducers were usually replaced by noninvasive contact or isotonic transducers. In the present anemometric study I used the isotonic transducers in simple "pulling" version with the sensitive membrane attached to the tip of the abdomen. This served as an auxiliary detector for monitoring all relative changes in hemocoelic pressure, *e.g.*, inspirations, expirations, hermetical closure of all spiracles, or extracardiac pulsations in hemocoelic pressure.

The microanemometer setup for monitoring the passage of air through the spiracles consisted of four independent thermographic channels (4 resistant bridges). Each channel contained two matched thermographic elements (thermistors) connected in the neighboring branches of the Wheatstone's resistant bridge. The thermistors (type 10 NR 17A, Pramet Co., Šumperk, Czechoslovakia) were positioned within the transducer so that exactly half of their bodies protruded from the inside end of the anemometric tubing (O.D. of the selected thermistors was less than 300 μm, resistance from 280 to 480 Ohm) (see Fig. 1A). The movement of air in the tubing in either direction caused positive or negative temperature changes on slightly warmed body of the thermistor. This resulted in the respective + or − imbalance of the resistance bridge (bridge feeding 2 V AC of 5 kHz). After amplification and decoding of the signal, the recorder showed the respective + or − deviations from the midline. The two thermistors paired in one channel recorded the + and − changes with reversed polarity. When only one was connected to the spiracle while the other remained free as a control, the instrument selectively recorded inspirations and expirations in the opposite directions. This type of selective recording from up to four spiracles was predominantly used with developing pupae where inspirations alternated with frequent expirations (Figs. 1A, B).

The "nonselective" type of anemometric measurements involved situations when both thermistors of each pair terminated on the spiracles. In this case, each thermistor of the pair recorded movements of the same polarity (such as inspiration) in opposite directions from the midline. The responses were reversed with the reversed (expiratory) directions and, obviously, atypical or aberrant response occurred when both thermistors of a pair became activated at the same moment. In this way it was possible to record simultaneously from up to eight spiracles, provided that the polarity of responses was known (otherwise an inspiration in the + gate could be confused with
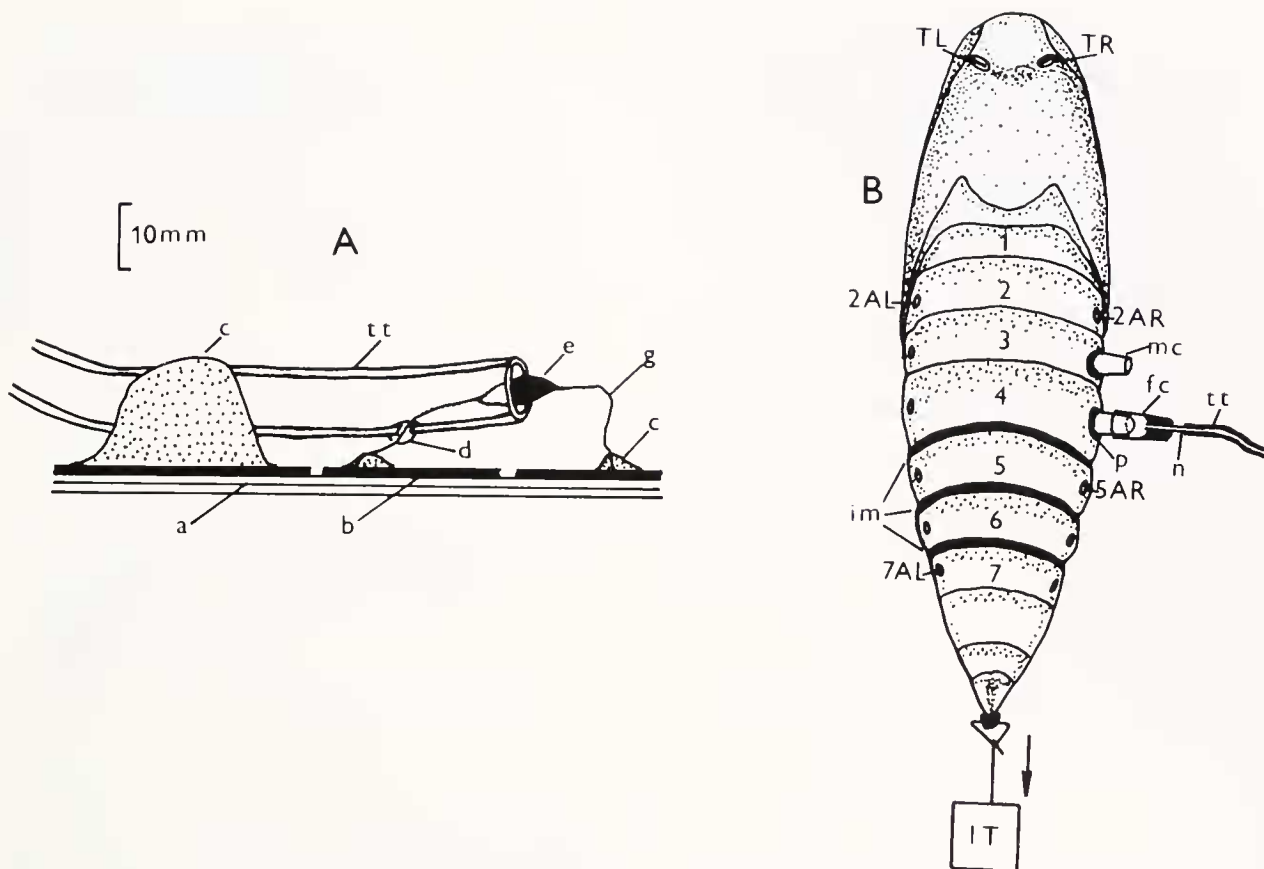
Figure 1. A—anemometric transducer formed by one thermistor (e) placed in the orifice of teflon tubing (tt) leading to the spiracle. B—a pupa with the system of spiracle identification, showing connectors of the anemometer tubings and the way of an isotonic transducer (IT) attachement. Legend: a—plastic plate of the printed circuits, b—etched circuitry, c—soldering, d—passage of thermistor wire through the wall of the tubing, e—thermistor, fc—female connector, g—silver wire of the thermistor used for positioning its body in the middle of the tubing section, im—intersegmental flexible membranes, mc—male connector permanently fixed around spiracles, n—stainless steel needle, p—paraffine wax seal, tt—teflon tubing.

an expiration in the − gate). Therefore, the anemometric measurements were always coupled with the direct or indirect transducers of hemocoelic pressure. This enabled clear distinction between the movement of air inside or outside of the body. A few examples of the combined anemo-tensiometric recording of the described type are shown in Figures 2 to 5.

The body of the anemometric transducer consisted of a printed circuit plate with the thermistors, resistors, and outlet cables, forming four complete thermographic units. It was maintained constantly within a thermostatted plastic box at 27°C ± 0.1°C. Details of manufacture, calibration, and performance of the instrument will be described in detail elsewhere (Sláma, 1988a). Measurements were made on a four-channel tensiometric unit M-1000 (Mikrotechna Co., Praha, Czechoslovakia) and a battery of linear recorders. Details of this electronic

setup were described earlier (Sláma, 1984a, 1984b). The instrument was sensitive to a few nl of air movement; the frequency resolution was better than 4 Hz. The sample records shown in Figures 2 to 5 were selected from measurements on at least five pupae in each species.

Results

Respiration of developing adults of Actias selene

Pupae of the Chinese moon moth (*Actias selene*) develop without diapause. Preliminary recordings showed that they are breathing most of the time through the largest spiracles, located on the third abdominal segment. About midway in the pupal-adult transformation there are 30 to 40 min periods of relative ventilatory rest (at 25°C). The hemocoelic pressure shows slightly subatmospheric values (−300 to −500 Pa) and air is constantly

sucked in through one largely constricted spiracle (most commonly one of the thoracic or third abdominal). These resting periods regularly alternate with 15 to 20 min periods of rhythmic pulsations in hemocoelic pressure. The pulsations are extracardiac. They are caused by contractions of the intersegmental abdominal muscles, the heart-beat operates independently on two different frequencies and causes 100-times smaller changes in hemocoelic pressure.

Figures 2A and B show ventilatory functions of three selected spiracles during the terminal part of one extracardiac pulsation. The lowest trace is an auxiliary record obtained from the isotonic transducer attached to the tip of the abdomen. This record shows the frequency of the extracardiac hemocoelic pulsation to vary from 26 to 18 strokes per min, and the amplitude of the associated abdominal movements from 5 to 15 μm. Another instructive feature of the record is the intervals when all spiracles are hermetically sealed (indicated by horizontal lines at the bottom of Fig. 2A and B). During this time, hemocoelic pressure decreases and the abdomen contracts with constant velocity of 84–90 μm · min^{-1}, which is proportional to the O_2 consumption rate. A further characteristic of the record is large inspirations of air (indicated by triangles). These are connected with a sudden increase of internal body volume, elevation of the subatmospheric hemocoelic pressure and, finally, with sudden elongation of the abdomen. This has been recorded by the transducer in Figures 2A and B.

The above relationships suggest that the record from the isotonic transducer can reveal many details related to the respiration dynamics of the investigated pupa. However, it does not show which of the spiracles was functioning. This information is partly provided by the anemometric records in Figures 2A and B. The spiracles on the third abdominal segment are most frequently used in this species. Each of the spiracles measured can function independently. Thus, the left thoracic spiracle (TL) was hermetically closed at the beginning of recording, it was slightly opened but held considerably constricted between the third and fifth min, and it opened after both 3A spiracles were tightly closed (Fig. 2B). This experiment does not determine how much the thoracic spiracular valves were opened during the maximum amplitude of the anemometric responses. It is also unknown whether some of the remaining intact spiracles would flutter at the same time. Nevertheless, the amplitudes showing the movement of up to ±0.5 μl of air across the spiracular valve with every stroke of the intersegmental muscles (Figs. 2A, B) provide clear experimental evidence that hemocoelic pulsations may indeed cause a very efficient tracheal ventilation.

The comparison of anemometric records from the right and left 3A spiracles documents that each spiracle
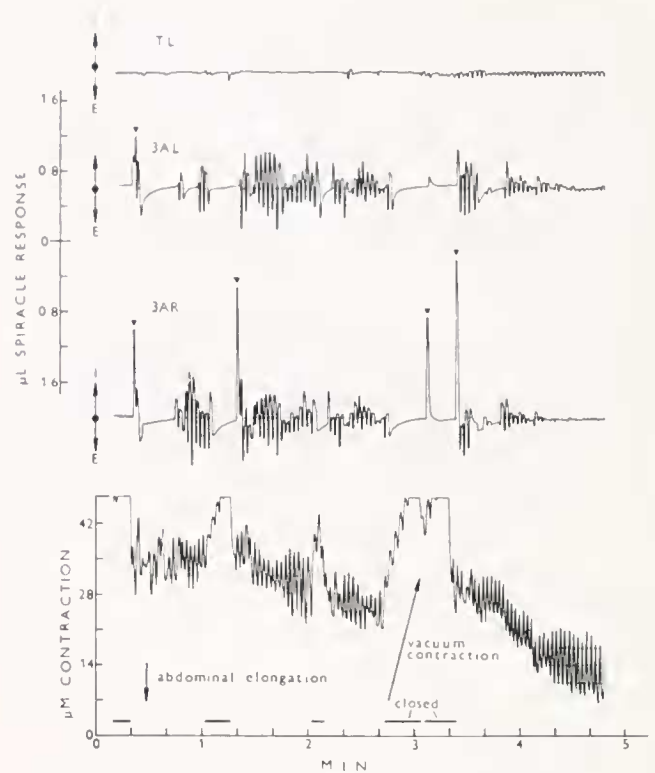

Figure 2A. *Actias selene*, midway during the pupal-adult transformation. Example of the selective recording of inspirations (I) and expirations (E) from three spiracles (TL-left prothoracic, 3 AL-left and 3 AR-right 3rd abdominal) during the second half-period of an extracardiac pulsation. Each of the three anemometric channels had one "active" thermistor connected with spiracle while the other was free. Lower trace shows relative changes of internal volume and hemocoelic pressure, recorded indirectly via an isotonic transducer attached to the tip of the abdomen. The heavy black lines on the bottom indicate periods when all spiracles are hermetically closed; triangles show larger inspirations of air.

can instantly open or close in full synchronization with individual strokes of the hemocoelic "hydraulic bellows." The frequency of 0.3 to 0.5 Hz (one stroke in 2–3 s) is sufficiently low to allow such synchronization. Although the 3AL and 3AR spiracles function in concert for some time, certain strokes are missing on one or another trace. Moreover, larger inspirations of 1.0 to 1.5 μl of air were realized selectively by a sudden 300 ms flutter of the 3AR valve, while the contralateral valve remained silent. These results show that functioning of the spiracular valves is controlled by a nervous system whose functions are precisely coordinated with nervous control of the extracardiac pulsations.

Respiration of diapausing cecropia pupae

Hyalophora cecropia invariably enters a prolonged pupal diapause which persists for at least 6 months at room
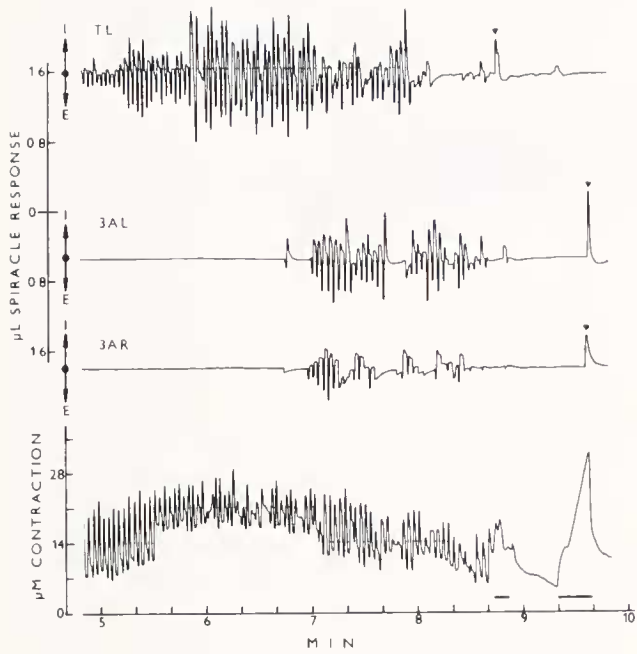
Figure 2B. Continuation of the recording from Figure 2A showing the terminal part of the extracardiac pulsation including ventilation of the left prothoracic spiracle.

temperature. In this case, anemometric recordings were preceded by one or more days of continuous monitoring of respiratory dynamics using the isotonic transducer alone (at 25°C). This was necessary to recognize possible abnormalities in respiratory functions resulting from the attachment of spiracles to anemometric tubing. The anemometric technique permits unrestrained movement of air across the spiracles. However, the records occasionally signaled suffocation or incomplete CO_2 ventilation after prolonged anemometric recordings. Usually this was recognized by supernumerary, out-of-schedule pulsations. In this case the connectors were dismantled for some time. In the majority of diapausing Cecropia pupae (15 specimens; some of them measured several times) there were regular bursts of CO_2 release at 5 to 7 h intervals. In addition, there were brief expiratory outbursts of intratracheal gases associated with abdominal rotation once per 12–16 h.

Figure 3 shows a typical sample of the combined tensio-anemometric recording during the interburst period. The lower trace from the isotonic transducer reveals relative changes of internal body volume. It shows that the spiracles were hermetically sealed most of the time (internal pressure was subatmospheric throughout). The closure is indicated by the periods when the abdomen retracts due to decreasing pressure with a constant speed of 3 μm per min. Volumetric calibration of this pupa under water revealed that 1 μm of abdominal contraction was

equivalent to 240 nl of internal volume. Thus, isotonic transducer can be used as a rapid and simple detector of O_2 consumption rate. The constant rate of 3 $\mu m \cdot min^{-1}$ of abdominal retraction in Figure 3 corresponded to O_2 consumption of 720 $nl \cdot min^{-1}$ (43.2 $\mu l\ O_2 \cdot h^{-1}$).

The diapausing Cecropia pupae maintain subatmospheric hemocoelic pressure during the whole interburst period. Except for the CO_2 burst, they perform mechanical expiration only during a brief rotational response once per 12–16 h. In such prolonged inspirations, which is very common in all diapausing lepidopteran pupae, the anemometric network can be used for simultaneous recording from eight spiracles, as shown in Figure 3. In the upper part we find four anemometric traces corresponding to four pairs of eight reciprocal gates. Each trace is thus common to two spiracles whose inspirations are displayed in the opposite directions (see arrows in
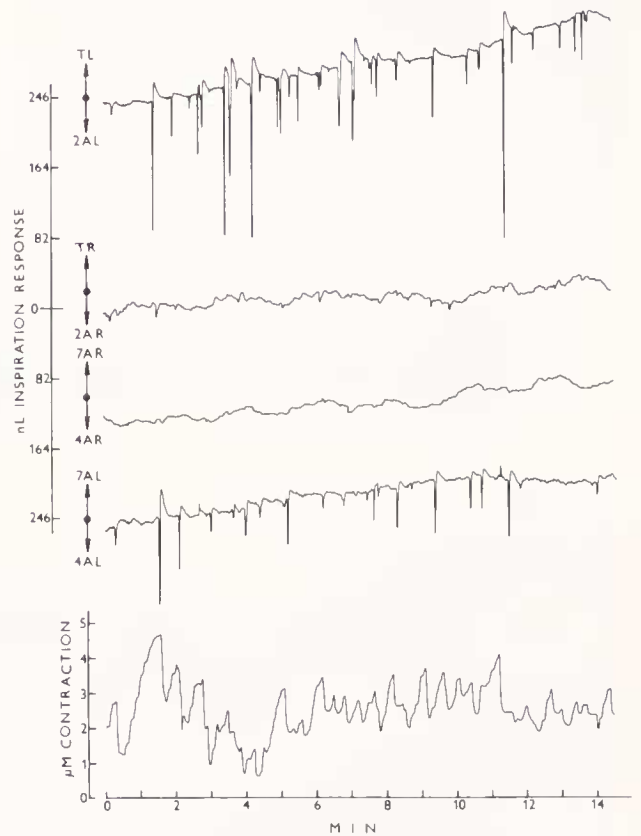

Figure 3. *Hyalophora cecropia,* diapausing male pupa during the interburst period (25°C). Recording of inspirations from eight spiracles (T-thoracic, AL-left, AR-right abdominal spiracles). Note that in contrast to single gate operation as shown in Figure 2, the two reciprocal gates of each channel record here inspirations in the opposite direction from the midline (see arrows). Lower trace from the isotonic transducer reveals intervals and magnitude of all inspirations, *i.e.,* sudden abdominal elongation. Hermetical closure of spiracles between inspirations is manifested by a steady upward movement of the pen driver (abdominal contraction due to decrease of hemocoelic pressure).

Fig. 3). The intervals and magnitudes of all inspirations in the body can be checked on lower trace from the isotonic transducer, as explained above. Accordingly, any sudden expiration of intratracheal gas, if present, should be recognized easily by instantaneous abdominal contraction, whereas passive inspirations are caused by abdominal elongation (for more details see Sláma, 1984a).

Figure 3 shows that the pupa used only two spiracles for periodic inspirations during the interburst period, *i.e.*, second and fourth left abdominal. Their function was coordinated with an accuracy of a few ms. With values of internal pressure ranging from -300 Pa to -2 kPa, the 2 AL spiracle showed larger inspirations up to 200 nl of air, while the 4 AL gave smaller and more variable responses of 20 to 50 nl at a time. This suggests that the aperture of each spiracle can be individually controlled. The general respiratory pattern of this pupa was that air was taken in discontinuously in sudden gulps lasting only 100–200 ms at more or less regular intervals of 3 to 4 per min. The intervals between inspirations could be prolonged by decreasing ambient temperature. For example, at 15°C the intervals were approximately twice as long as at 25°C. Near the CO_2 burst period, the passive respiratory movements often disappeared from the records though hemocoelic pressure remained slightly below barometric level. Gentle touching of the surface (causing small volumetric changes within the pupal body) always evoked an immediate anemometric response in one or both thoracic spiracles. This suggests that some spiracles can be maintained constricted, allowing a constant inflow of 720 nl·min^{-1} of air into the body.

Management of CO_2 by diapausing Cecropia pupae

Figure 4 shows the anemometric responses during the whole period of CO_2 burst in the same pupa used in Figure 3. The bottom trace from the isotonic transducer shows rather delicate extracardiac hemocoelic pulsation with an amplitude of only about 1 μm of abdominal movement and a frequency of 21–23 strokes per min. In principle, the movement of flexible abdominal segments acts bellows-like on the tracheae and produces the inflow or outflow of gas through any open spiracle. The amplitudes of the anemometric responses are directly proportional to the aperture of the spiracular valve. For example, a completely closed spiracle gives no response, a partly constricted one gives an intermediate response, and a fully opened spiracle should give the maximum response. In addition, amplitudes of the individual anemometric responses are weakened by an increasing number of spiracles that open simultaneously.

Some of the above outlined relationships are illustrated by the upper traces in Figure 4. The "nonselec-

tive" variant of the anemometric recording and slow chart speed do not show which of the two spiracle mates on each channel have actually responded. The arrangement of the pairs of spiracles shown in Figures 3 and 4 was made after the foregoing finding that the two spiracles of a pair did not function at the same time. Figure 4 shows that some spiracles, such as 7 AR and 4 AR remained closed throughout almost the entire period of the CO_2 burst. It also shows that the "master spiracles" from Figure 3 (2 AL and 4 AL) were probably functional during the initial half-period of the burst, whereas a larger thoracic spiracle (TR) opened at the end of the burst. Different amplitudes of the anemometric responses and permanent closure of some spiracles suggest that the spiracles cannot be maintained widely opened by high CO_2 concentration during the burst, as has been generally believed. In reality, some spiracles can be selectively ventilated at different sites and at determined periods of the CO_2 burst. The records of other burst periods in this and other pupae indicated that the pattern in Figure 4 is variable. This suggests that alteration of the spiracle opening sequence is under non-stereotyped physiological control.

Pupae of Cecropia and some other saturniids usually close all spiracles for 10 to 20 min after termination of a CO_2 burst, when internal pressure is close to atmospheric level. During this period, abdominal segments retract with the velocity of 5 to 12 μm·min^{-1} and hemocoelic pressure declines to -5 kPa or less. Such a large vacuum inside the body becomes sequentially reduced to the usual values by large inspirations of air, most frequently through thoracic or last abdominal plus thoracic spiracles. Sometimes more than 20 μl of air are taken in a single surge and sometimes more than 0.5 ml of air is taken in during one min. The process continues until hemocoelic pressure becomes adjusted to about -500 Pa. This is followed by the type of respiration as shown in Figure 3.

Respiration of diapausing Sphinx ligustri

Sphingid pupae typically show regular periods of inspirations that give the records of hemocoelic pressure a saw-tooth appearance. The records from isotonic transducers are mirror images of changes in hemocoelic pressure (see Sláma, 1984a), *i.e.*, the abdomen contracts slowly when hemocoelic pressure passively decreases. The bottom trace in Figure 5 shows the saw-tooth pattern in *Sphinx ligustri*. The teeth indicate intervals and size of the inspirations. A further peculiarity of sphingid pupae is the slowly expanding internal volume (visible as the slow decline tendency of the lower trace in Fig. 5). This is terminated once per several hours by a large expiration associated with abdominal rotation or, eventually, by a CO_2 burst. Useful information from this record are
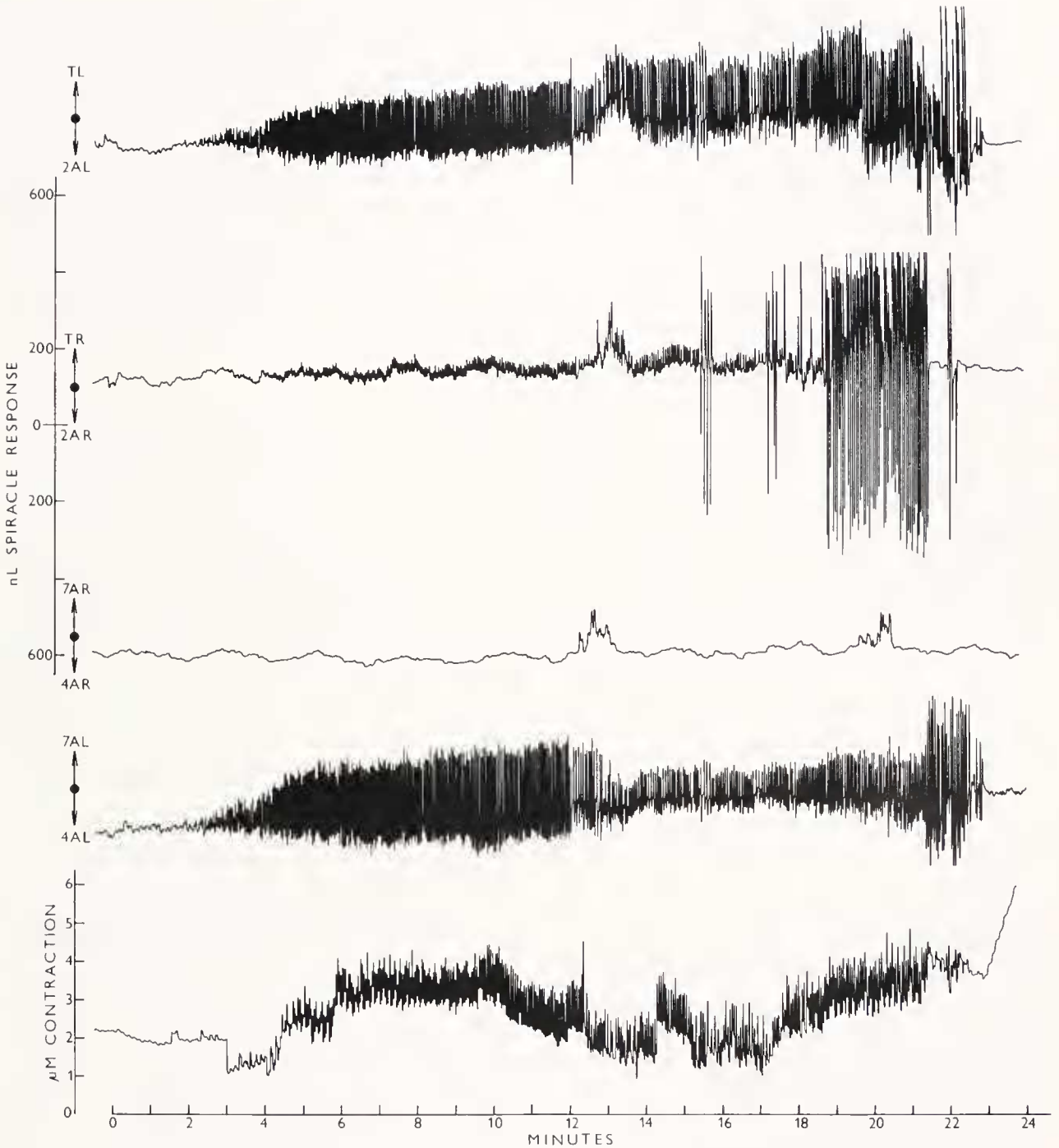
Figure 4. *Hyalophora cecropia,* the same preparation as in Figure 3, recording from eight spiracles during the 20-min period of CO_2 burst associated with an extracardiac pulsation (25°C). Each of the four anemometric traces gives unresolved responses from four pairs of spiracles (arrows indicate inspirations in the particular spiracles, but they may be expirations in the respective counterparts). The amplitudes of the anemometric responses are proportional to the degree of opening of the spiracular valves. Lower trace shows the bellows-like ventilatory movements of the distal abdominal segment.

more or less regular intervals of inspirations at 30 s, and the velocity of the constant abdominal contraction of 2.5 $\mu m \cdot min^{-1}$. Calibration of the pupa under water revealed

that 1 μm of abdominal contraction was equivalent to 110 nl of air transported through the spiracle or 110 nl of O_2 consumed (O_2 consumption of 16.5 $\mu l \cdot h^{-1}$).
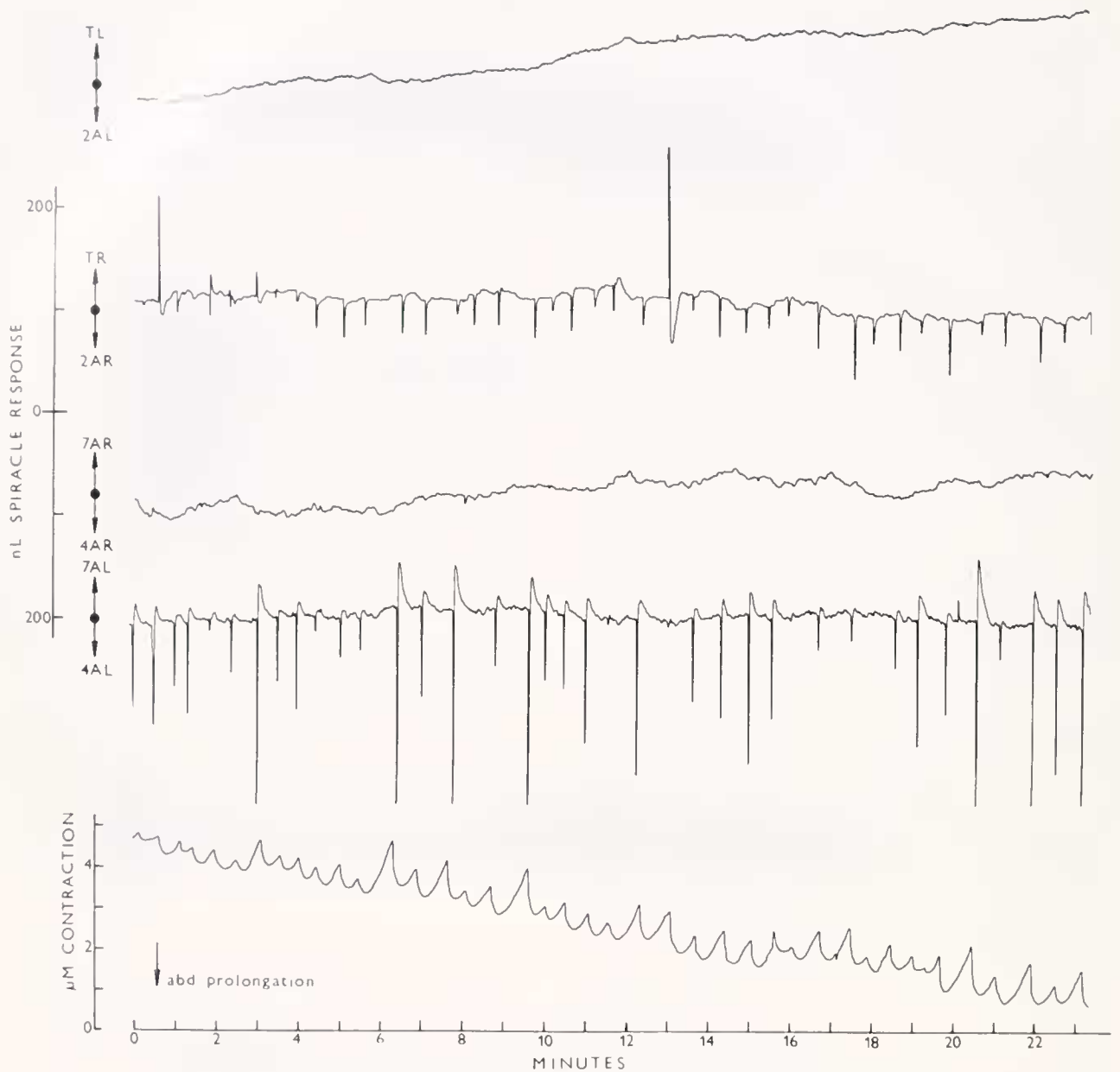
Figure 5. *Sphinx ligustri*, diapausing pupa. A sample from prolonged recordings between the CO_2 bursts during the period of discontinuous inspirations of air. Lower trace shows the characteristic "sawtooth" pattern of hemocoelic pressure changes revealed indirectly by an isotonic transducer from the tip of the abdomen. Inspirations are indicated by sudden abdominal prolongation (sudden decrease of the internal vacuum). The anemometric traces show inspirations in eight spiracles indicated by the arrows. Note that only 4 AL and 2 AR spiracles were used for inspirations, the 1 R spiracle was used only twice, while the rest of spiracles were hermetically closed all time.

The anemometric traces (Fig. 5) document that this pupa also used inspirations through selected abdominal spiracles. Inspirations occurred only in 4 AL assisted by 2 AR. A sudden inspiration through the right thoracic spiracle occurred as the two abdominal spiracles go silent (around 13:00 min recording time). Such swift interplay between close or more distant spiracles is quite common.

Direct evidence that it was not expiration through the paired 2 AR spiracle is provided by the tensiometric record below, which shows abdominal elongation due to volumetric increase, not contraction.

The pattern of inspirations in Figure 5 shows that the "master" and assisting spiracles taking part in discontinuous air intake are not only located on different body

segments, but can occur on contralateral sides. More-over, the two functioning spiracles opened for a total of only 7 s of 22 min, while all other spiracles were hermetically closed. This pattern when all spiracles are closed while only two of them would flutter for no more than 0.53 per cent of time seems to be a common feature in diapausing pupae of Lepidoptera. It provides a serious argument against the belief that the pupa could breathe by simple diffusion of respiratory gases through spiracles. The situation in Fig. 5 cannot be taken as a stereotypic model for all pupae of a species. Other pupae of *S. ligustri* did not use 4 AL as the most active spiracle. Some used preferentially TR, 3 AL, or 7 AL.

The tobacco hornworm Manduca sexta

Large sphingid pupae (*Acherontia atropos, Herse convolvuli, Manduca sexta*) show very special respiratory scenarios during diapause. They maintain an internal vacuum and tend to use a single "master" spiracle for prolonged discontinuous inspirations, while all other spiracles are tightly closed. This often continues unaltered for periods of more than 15 h at room temperature or for several days at 5°C. Figure 6 shows a 24-min segment taken from an uninterrupted 48-h recording in diapausing *Manduca*. The bottom trace comes from the isotonic transducer. It shows regular inspirations at 1.5 to 2 min intervals. The inspirations have been associated with sudden increases of internal body volume which is manifested on the record by sudden elongations of the abdomen. After termination of the anemometric measurements, the hemocoel cavity of this pupa was connected with the hydraulic transducer for calibration of the system. The bottom trace in Figure 5 appeared as a mirror image of changes in hemocoelic pressure (for more details see Sláma, 1984a).

The records in Fig. 6 show that, of eight spiracles, the pupa inspired only through the left thoracic spiracle. All others were kept hermetically closed. Each inspiration lasted approximately 200 ms, the anemometer detected a rapid flow of 600 nl of air. Further measurements on this and other diapausing *Manduca* pupae revealed important biophysical data that can be summarized as follows: (a) the velocity of the steady abdominal contraction is 2 μm·min^{-1}; (b) 1 μm of abdominal movement corresponds to 2.9 Pa change in hemocoelic pressure; (c) 1 μm of abdominal movement is equivalent to 150 nl of air inspired or O_2 consumed, and (d) the baseline hemocoelic pressure is 0.8 kPa under atmospheric level.

The above data reveal a hitherto unknown homeostatic mechanism. This mechanism regulates a constant body length within the limits of ± 5 μm (*i.e.,* 1/10000th of pupal length), maintains more or less constant body volume within the limits of ± 750 nl (*i.e.,* 1/13000th of

body volume), or regulates hemocoelic pressure within the limits of ± 14.5 Pa (*i.e.,* 1.4 mm hydrostatic pressure). This illustrates remarkable accuracy in the underlying sensory and neurophysiological mechanisms.

Extensive anemometric studies with diapausing pupae of various lepidoptera consistently revealed subatmospheric hemocoelic pressures and predominantly closed tracheal systems. Air was mechanically sucked into the body whenever some spiracle opened while mechanical expiration was unusually rare. In certain cases, as in the pupa of *Manduca,* body volume remained constant for many hours in spite of a well-documented net inflow of air into the otherwise hermetically sealed pupal body. This effective nitrogen concentration within the closed pupal case has been studied intensively. Details will be described elsewhere.

Discussion

The mechanics of insect respiration and tracheal ventilation were studied some 80 years ago (for review see Babák, 1912). About 30 years ago, the discontinuous respiration of insects became a favorite subject of insect physiology (Punt, 1950; Schneiderman and Williams, 1955; Buck, 1962; Keister and Buck, 1964). Though it still is a favorite subject of more recent reviews (Miller, 1981; Kestler, 1985), few additional insights have been added since Schneiderman and his co-workers defined changes in intratracheal pressure, described the microcycles of suction respiration, and explained the basic mechanisms of spiracular functions (Levy and Schneiderman, 1966; Brockway and Schneiderman, 1967; Burkett and Schneiderman, 1966). The present study stems directly from these publications and confirms most, though not all, of the conclusions.

Information is available on neuromuscular control of spiracular functions and ventilatory movements (review by Miller, 1981). Anemometric techniques, combined with the direct or indirect detectors of hemocoelic pressure, now permit monitoring the actual passage of respiratory gases through individual spiracles. These techniques, in combination with very sensitive microrespirographic methods (see Sláma, 1984b), help to obtain simultaneous monitoring of the course of O_2 consumption, internal volume, and hemocoelic pressure changes and, most importantly, monitoring of the functioning spiracles (for more technical details see Sláma, 1984a, 1984b, 1988a).

August Krogh's pioneering work led to a theory of purely diffusive gas transfer within the tracheal system (Krogh, 1920). This model was subsequently corroborated by Weiss-Fogh (1964) who concluded that there were no reasons to assume mechanisms for insect respiration other than simple gaseous diffusion. The model
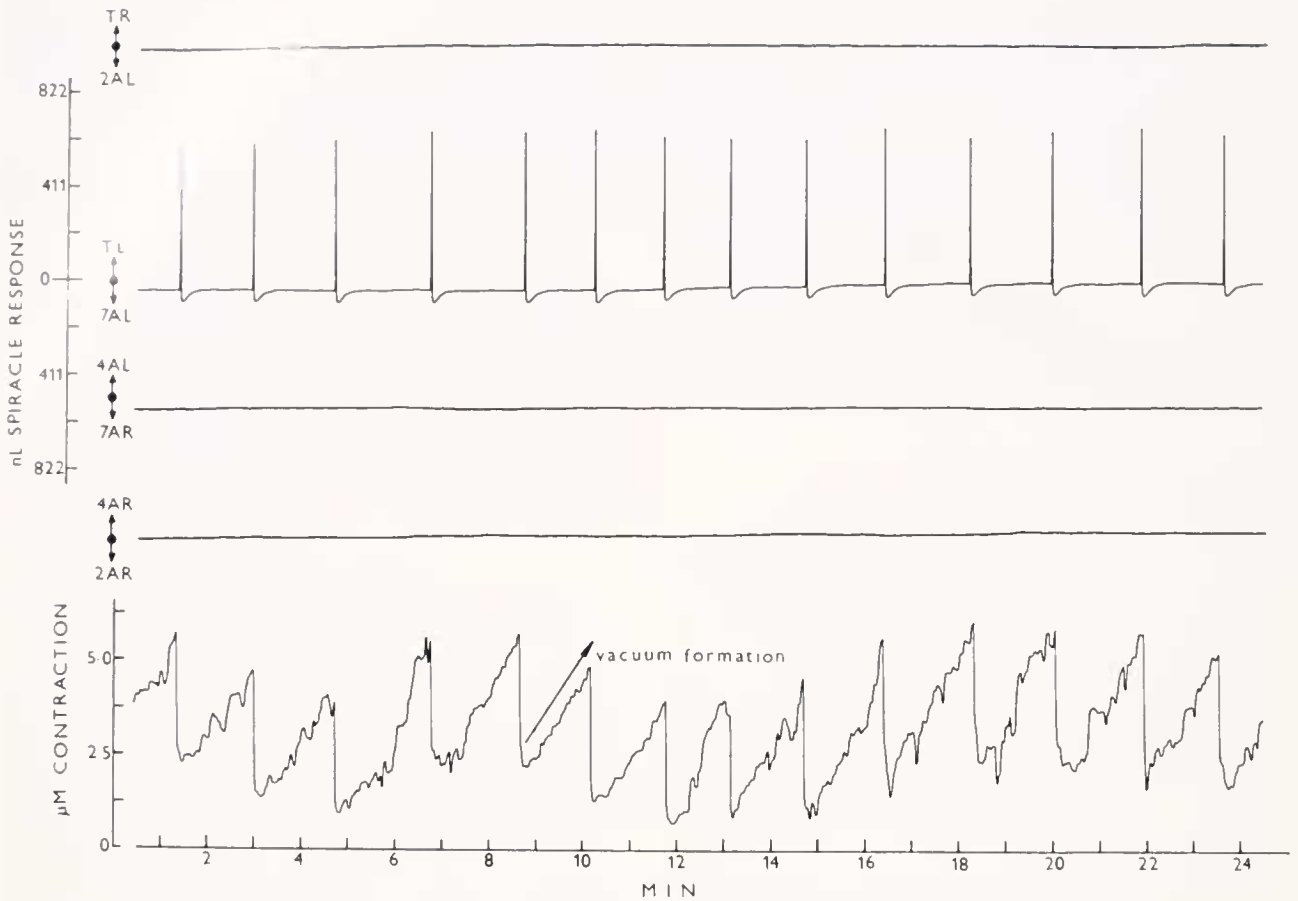
Figure 6.   *Manduca sexta*, diapausing female pupa. Recording of regular inspirations from eight spira-
cles during the interburst period. Only left prothoracic spiracle was functional while all others were tightly
sealed. The bottom trace from the isotonic transducer displays the associated pressoric or volumetric
changes from movement of the tip of the abdomen.

was thoroughly analyzed by Buck (1962) and recently
updated by Kestler (1985). The most important chal-
lenge to the diffusion theory has hitherto been the results
of Hazelhoff. The latter's work is known mainly from the
description of Prof. H. Jordan (see Jordan, 1927). Hazel-
hoff found that insect spiracles were completely closed
most of the time—a finding that few have recognized but
which is fully confirmed in the present study.

The diffusion theory of insect respiration was ques-
tioned in various review articles (Chauvin, 1949; Kuz-
netzoff, 1953; Buck, 1962; Miller, 1981; Kestler, 1985),
but the critiques lacked experimental data. The original
reasonings of Krogh (1920) supporting the purely diffu-
sive mode of insect respiration were based on the premise
that immobile stages of insects did not show ventilatory
movements. However, we see (Figs. 1A, B and 3) that
immobile pupae do exhibit minute hemocoelic pulsa-
tions. These result in active tracheal ventilation. Buck
(1962) postulated that a convective stream of intratra-
cheal gas can be achieved by extremely small changes in

internal pressure. Indeed, the changes in mechanical
pressure associated with the described pulsations are so
small (sometimes less than 5 Pa or less than 1 μm of in-
tegumental movement) that they can be visualized only
via recently available electronic devices. This may ex-
plain why the existence of extracardiac pulsations in
hemocoelic pressure remained unknown until 1976
(Sláma, 1976). Recent investigations show that the pul-
sations are present everywhere. They occur, for example,
in immobile prepupae and in pupae of all major endo-
pterygote groups, including Coleoptera, Lepidoptera,
Hymenoptera, and Diptera (Sláma, 1984a). The wide-
spread occurrence of these pulsations (ventilatory move-
ments) in the immobile stages with low metabolic rates
provides strong circumstantial evidence that simple
diffusion principles are not satisfactory for the transport
of gases through spiracles. However, the diffusion princi-
ples formulated by Krogh (1920) may find practical use
for the internal transport of O_2 between tracheae and tis-
sues. This view is consistent with the calculations of Buck

(1962), and of Kestler (1985), as well as with the recent views concerning respiratory functions of insect tracheoles (Wigglesworth, 1984).

To illustrate some arguments against the role of diffusion in the exchange of O_2, N_2, and CO_2 between the pupae and the environment, we may discuss again the case of *Manduca* in Figure 6. Here the unidirectional suction stream of air first passes through a narrow spiracular sieve into a cavity above the spiracular valve. The valve flutters only in the left thoracic spiracle for 100–200 ms about once per minute. Thus it opens only for approximately 0.5% of the time. During its opening, air is propelled inside vigorously by a 0.5 to 0.8 kPa pressure difference (according to preliminary calculations, the speed of the air stream is close to 20 m·s^{-1}). All other spiracular valves are permanently and hermetically sealed for several hours, and at lower temperatures (5–10°C) they may be closed for several days. For obvious reasons, it is unrealistic to expect diffusion of the respiratory gases through just one spiracle that is sealed 99.5% of time and whenever it opens there is a fast stream of air.

The respiration pattern of *Manduca* is not restricted to the large-sized sphingid pupae. It is quite common among diapausing pupae in a number of lepidopteran families, including miniature pupae of Geometridae, where diffusion principles in respiration would be most likely. Reasons why lepidopteran pupae must live with closed spiracles, are still unknown. According to the literature (Buck, 1962; Kestler, 1985), the principal reason is water conservation.

The foregoing facts strongly argue that separate zones of the tracheal system can be ventilated by selective opening of the determined spiracles. Actual ventilation is brought about by genuine pulsations in hemocoelic pressure. These are generated in the majority of insect groups by contractions of the intersegmental muscles of the abdomen. Usually utilization of O_2, fixation of CO_2 in buffers, and hermetical closure of the spiracles, create subatmospheric pressures which are automatically conveyed to the gas-filled tracheae. An instantaneous inflow of fresh air occurs whenever a spiracle opens. This brief recapitulation of the observed respiratory relationships suggests that insects possess a neuromuscular mechanism for controlling inspirations and expirations through individual spiracles. The nervous system controlling opening or closing the spiracles has apparent motor outflow via a nerve system regulating the intersegmental muscles of the abdomen and, thereby, the hemocoelic pressure.

In *Tenebrio, Galleria,* and some other insects an autonomic (brain independent), parasympathetic-like nervous system regulating hemocoelic pressure has been described (Sláma *et al.,* 1979; Sláma, 1986). More recently,

this mechanism has been termed the coelopulse system (from the Greek *koiloma* or Latin *coelom* for cavity and *pulsus* for beating or striking). It regulates certain homeostatic functions in reproducing adults of various insect groups (Sláma, 1988b). There is increasing evidence that insect respiration is regulated by the same coelopulse system that regulates hemocoelic pressure. The mechanism mutually determines the duration of the pressure pulsations, controls the level of the baseline hemocoelic pressure, and regulates the intervals of inspirations (see Sláma, 1984a). The present anemometric data show that it may also control the function of individual spiracles. Thus, the coelopulse mechanism of insect respiration is composed of two elements: (a) neuromuscular system regulating the opening or fluttering of spiracular valves (metameric system of unpaired central and transverse nerves innervating the spiracles, with the adjacent perisympathetic neurohaemal organs), and (b) what may be termed "hydraulic bellows" driven by the intersegmental muscles of the abdomen with nerve impulses coming from the thoracic ganglia (generating changes in hemocoel pressure that force the air in or out through the selected spiracle). These complex physiological functions can be compared to playing an accordion. There are two interconnected nerve functions: one is responsible for pulling the bellows and the other for pressing the right keys on the keyboard. We know the instrument but we must now learn to listen to the melody of different insects.

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